BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

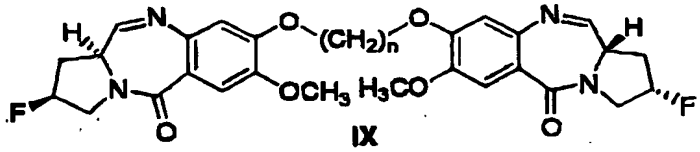
As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

435/NF/03

C2-FLUORO PYRROLO[2,1-c][1,4]BENZODIAZEPINE DIMERS

Field of the invention

The present invention relates to novel 2-fluoro-pyrrolo[2,1-*c*][1,4]benzodiazepine dimers useful as potential antitumour agents. The present invention also relates to a process for the preparation of novel 2-fluoro-pyrrolo[2,1-*c*][1,4]benzodiazepine dimers useful as potential antitumour agents. The present invention particularly relates to a process for the preparation of new bis-2-fluoro-pyrrolo[2,1-*c*][1,4] benzodiazepines useful as anticancer agents. More particularly, it provides a process for the preparation of 1,1'-{[(bisalkane-1,N-diyl)] dioxy} bis [(11a*S*) – 2 – fluoro – 7 – methoxy - 1, 2, 3, 11a – tetrahydro – 5*H* –pyrrolo [2, 1-*c*] [1, 4] benzodiazepin-5-one, with aliphatic chain length variations for the compounds and also describes the anticancer (antitumour) activity. The structural formula of novel bis-2-fluoro-pyrrolo[2,1-*c*][1,4]benzodiazepine is as follows, wherein n= 3,4,5,6,7,8,9,10.



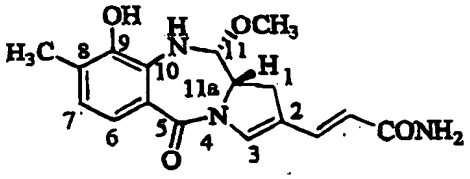
IX

Background of the invention

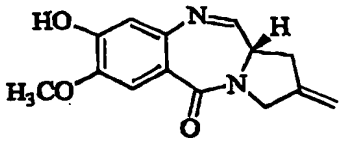
Pyrrolo[2,1-*c*][1,4]benzodiazepine antitumour antibiotics are commonly known as anthramycin class of compounds. In the last few years, a growing interest has been shown in the development of new pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs). These PBDs are a family of sequence selective DNA-binding antitumour antibiotics that bind exclusively to the exocyclic N2-guanine in the minor groove of DNA *via* an acid-labile aminal bond to the electrophilic imine at the N10-C11 position. (Kunimoto, S.; Masuda, T.; Kanbayashi, N.; Hamada, M.; Naganawa, H.; Miyamoto, M.; Takeuchi, T.; Unezawa, H. *J. Antibiot.*, **1980**, *33*, 665.; Kohn, K. W.; Speous, C. L. *J. Mol. Biol.*, **1970**, *51*, 551.; Hurley, L. H. Gairpla, C.; Zmijewski, M. *Biochem. Btophys. Acta.*, **1977**, *475*, 521.; Kaplan, D. J.; Hurley, L. H. *Biochemistry*, **1981**, *20*, 7572.) All biologically active PBDs possess the (*S*) configuration at the chiral C11a position which provides the molecule with a right-handed twist, which allows them to follow the curvature of the minor groove of B-form double-stranded DNA spanning three base pairs. Recently, PBD dimers have been developed that comprise two C2-exo-methylene- substituted DC-81 subunits tethered through their C-8 position via an inert propanedioxy linker. (Gregson, S. J.; Howard, P. W.; Hartely, J. A.; Brooks, N. A.;

Adams, L. J.; Jenkins, Kelland, L. R.; Thurston, D. E. *J. Med. Chem.*, **2001**, *44*, 737.). A recent development has been the linking of two PBD units through their C-8 positions to give bisfunctional alkylating agents capable of cross-linking DNA (Thurston, D. E.; Bose, D. S.; Thomson, A. S.; Howard, P. W.; Leoni, A.; Croker, S. J.; Jenkins, T. C.; Neidle, S.; Hurley, L. H. *J. Org. Chem.*, **1996**, *61*, 8141-8147). Recently, noncross-linking mixed imine-amide PBD dimers have been synthesized that have significant DNA binding ability and potent anti- tumour activitiy (Kamal, A.; Laxman, N.; Ramesh, G.; Ramulu, P; Srinivas, US Patent 636233; Kamal, A.; Ramesh, G.; Laxman, N.; Ramulu, P.; Srinivas, O.; Neelima, K.; Kondapi, A. K.; Srinu, V. B.; Nagarajaram, H. M. *J. Med. Chem.* **2002**, *45*, 4679.).

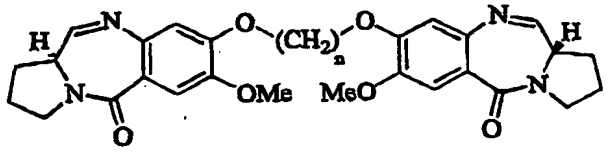
PBDs are of considerable current interest due to their ability to recognize and subsequently form covalent bonds to specific base sequences of double-stranded DNA. Naturally occurring pyrrolo[2,1-*c*][1,4]benzodiazepines belong to a group of antitumour antibiotics derived from *Streptomyces* species with family members including anthramycin, tomaymycin, sibiromycin, chicamycin, neothramycins A and B, and DC-81.



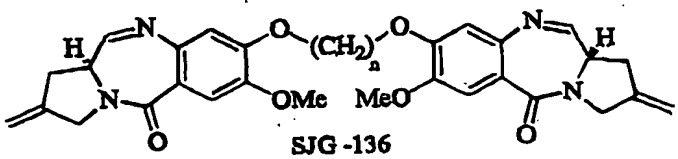
anthramycin



C2-exo-methylene-substituted DC-81



DC-81 dimers (n=3-5); DSB-120 (n=3)



SJG -136

However, the clinical efficacy for these antibiotics is hindered by several limitations, such as poor water solubility and cardiotoxicity and development of drug resistance and metabolic inactivation.

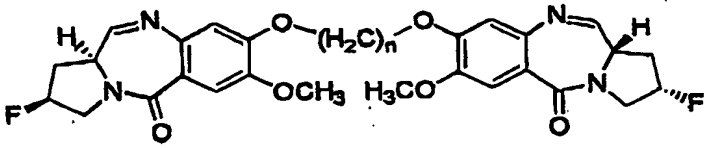
Object of the invention

The main object of the invention is to provide new bis-2-fluoro pyrrolo[2,1-*c*][1,4]benzodiazepines useful as antitumour agents.

Another object of the invention is to provide a process for the preparation of novel fluoro pyrrolo[2,1-*c*][1,4]benzodiazepines useful as antitumour agents.

Summary of the invention

Accordingly the present invention provides fluoro pyrrolo[2,1-*c*][1,4]benzodiazepine dimers of formula IX where n is 3 to 10.



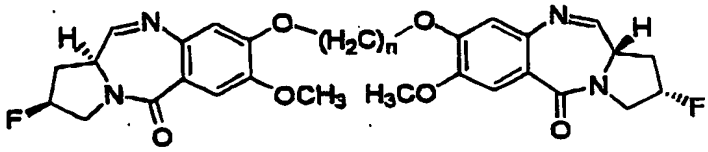
Formula IX

In one embodiment of the invention, the compound of formula IX is 1,1'-{[(propane-1,3-diyl)dioxy]bis[(11a*S*)-2-fluoro-7-methoxy-1,2,3,11a-tetra-hydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one]}.

In another embodiment of the invention, the compound of formula IX is 1,1'-{[(butane-1,4-diyl)dioxy]bis[(11a*S*)-2-fluoro-7-methoxy-1,2,3,11a-tetra-hydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one]}.

In another embodiment of the invention, the compound of formula IX is 1,1'-{[(pentane-1,5-diyl)dioxy]bis[(11a*S*)-2-fluoro-7-methoxy-1,2,3,11a-tetra-hydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one]}.

The present invention also provides a process for the preparation of bis 2-fluoro pyrrolo[2,1-*c*][1,4]benzodiazepines of formula IX



Formula IX

where n is 3 to 10, which comprises:

(a) reacting methyl (2*S*)-N-[4-benzyloxy-5-methoxy-2-nitrobenzoyl]-4-hydroxypyrrolidine-2-carboxylate dissolved in an organic solvent,

(b) cooling the solution and adding a solution of diethylaminosulfurtrifluoride (DAST) in an organic solvent drop wise;

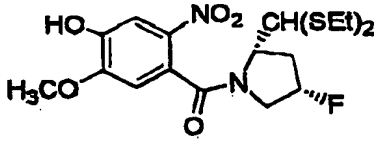
(c) isolating the methyl (2*S*)-N-[4-benzyloxy-5-methoxy-2-nitrobenzoyl]-4-fluoropyrrolidine-2-carboxylate with DIBAL-H formed in the presence of an organic solvent and cooling;

(d) isolating methyl (2*S*)-N-[4-benzyloxy-5-methoxy-2-nitrobenzoyl]-4-fluoropyrrolidine-2-carboxaldehyde formed;

(e) protecting methyl (2*S*)-N-[4-benzyloxy-5-methoxy-2-nitrobenzoyl]-4-fluoropyrrolidine-2-carboxaldehyde with EtSH in presence of an organic solvent;

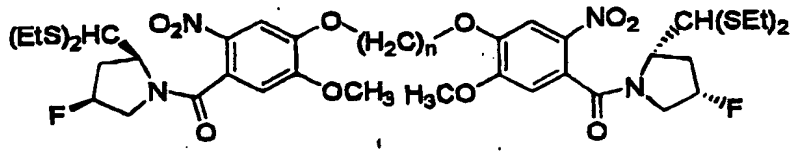
(f) isolating (2*S*)-N-[4-benzyloxy-5-methoxy-2-nitrobenzoyl]-4-fluoropyrrolidine-2-carboxaldehyde diethylthioacetal;

(g) reacting the (2*S*)-N-[4-benzyloxy-5-methoxy-2-nitrobenzoyl]-4-fluoropyrrolidine-2-carboxaldehyde diethylthioacetal with a debenzylating agent to obtain (2*S*)-N-[4-hydroxy – 5 – methoxy – 2 - nitrobenzoyl] – 4 – fluoropyrrolidine – 2 – carboxaldehyde – diethylthioacetal of formula VI,



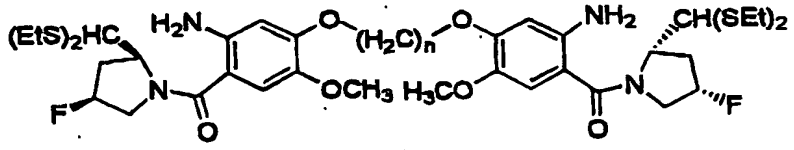
Formula VI

(h) reacting (2*S*)-N-[4-hydroxy-5-methoxy-2-nitrobenzoyl]-4-fluoro-2-carboxaldehyde diethylthioacetal of formula VI with a dibromoalkane in an aprotic water miscible organic solvent and in the presence of a mild inorganic base up to refluxing temperature and isolating 1,1'-{[(alkane-1,N-diyl)dioxy}bis[(2-nitro-5-methoxy-1,4-phenylene) carbonyl] bis [4-fluoropyrrolidin-2-carboxaldehyde diethylthioacetal] of formula VII where n is 3-10



Formula VII

(i) reducing the compound of formula VII with $SnCl_2 .2H_2O$ in presence of organic solvent up to a reflux temperature and isolating 1,1'-{[(alkane-1,N-diyl)dioxy}bis[(2-amino-5-methoxy-1,4-phenylene)carbonyl]]bis [4-fluoro-pyrrolidin-2-carboxaldehyde diethylthioacetal]] of formula VIII where n is 3-10



Formula VIII

(j) reacting the compound of formula VIII with a deprotecting agent to obtain bis 2-fluoro pyrrolo[2,1-c][1,4]benzodiazepines of formula IX wherein n is as stated above.

In one embodiment of the invention, the organic solvent used in steps (a), (b) and (c) comprises CH_2Cl_2.

In another embodiment of the invention, in step (a) the solution is cooled to a temperature of -78°C.

In another embodiment of the invention, the drop wise addition in step (b) is carried out for a period of 40 min.

In another embodiment of the invention, step (c) is carried out after 15 hours of step (b).

In yet another embodiment of the invention, the cooling in step (c) is done to a temperature of -78°C and for a period of 45 minutes.

In another embodiment of the invention, step (e) is carried out in presence of an organic solvent and at room temperature.

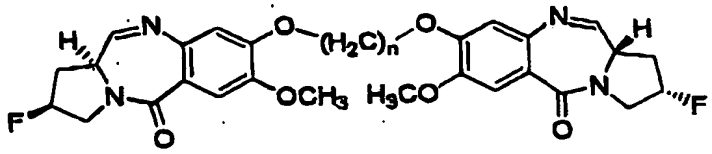
In yet another embodiment of the invention, the (2*S*)-N-[4-hydroxy-5-methoxy-2-nitrobenzoyl]-4-fluoro-2-carboxaldehyde diethylthioacetal of formula VI is reacted with a dibromoalkane in an aprotic water miscible organic solvent selected from the group consisting of acetone, acetonitrile and DMF and in the presence of a mild inorganic base selected from the group consisting of K_2CO_3, $CsCO_3$ and $BaCO_3$.

In another embodiment of the invention, step (h) is carried out for a period of about 48 hours.

In another embodiment of the invention, the reduction in step (i) is carried out in the presence of an organic solvent comprising methanol.

In yet another embodiment of the invention, the deprotecting agent comprises a combination of $HgCl_2$ and HgO in CH_3CN/H_2O.

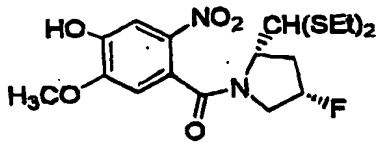
The present invention also provides a process for the preparation of bis 2-fluoro pyrrolo[2,1-*c*][1,4]benzodiazepines of formula IX



Formula IX

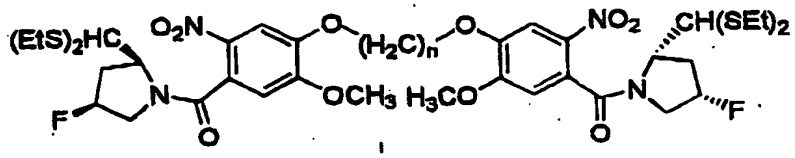
where n is 3 to 10, which comprises:

(a) (2*S*)-N-[4-hydroxy – 5 – methoxy – 2 - nitrobenzoyl] – 4 – fluoropyrrolidine – 2 – carboxaldehyde – diethylthioacetal of formula VI,



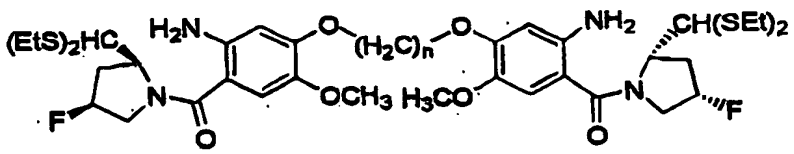
Formula VI

(b) reacting (2*S*)-N-[4-hydroxy-5-methoxy-2-nitrobenzoyl]-4-fluoro-2-carboxaldehyde diethylthioacetal of formula VI with a dibromoalkane in an aprotic water miscible organic solvent and in the presence of a mild inorganic base up to refluxing temperature and isolating 1,1'-{[(alkane-1,N-diyl)dioxy}bis[(2-nitro-5-methoxy-1,4-phenylene) carbonyl] bis [4-fluoropyrrolidin-2-carboxaldehyde diethylthioacetal] of formula VII where n is 3-10



Formula VII

(c) reducing the compound of formula VII with $SnCl_2 .2H_2O$ in presence of organic solvent up to a reflux temperature and isolating 1,1'-{[(alkane-1,N-diyl)dioxy}bis[(2-amino-5-methoxy-1,4-phenylene)carbonyl]]bis [4-fluoro-pyrrolidin-2-carboxaldehyde diethylthioacetal]] of formula VIII where n is 3-10



Formula VIII

(d) reacting the compound of formula VIII with a deprotecting agent to obtain bis 2-fluoro

pyrrolo[2,1-c][1,4]benzodiazepines of formula IX wherein n is as stated above.

In yet another embodiment of the invention, the (2*S*)-N-[4-hydroxy-5-methoxy-2-nitrobenzoyl]-4-fluoro-2-carboxaldehyde diethylthioacetal of formula VI is reacted with a dibromoalkane in an aprotic water miscible organic solvent selected from the group consisting of acetone, acetonitrile and DMF and in the presence of a mild inorganic base selected from the group consisting of K_2CO_3, $CsCO_3$ and $BaCO_3$.

In another embodiment of the invention, step (b) is carried out for a period of about 48 hours.

In another embodiment of the invention, the reduction in step (c) is carried out in the presence of an organic solvent comprising methanol.

In yet another embodiment of the invention, the deprotecting agent comprises a combination of $HgCl_2$ and HgO in CH_3CN/H_2O.

Detailed description of the invention

The present process provides a process for the preparation of bis 2-fluoro pyrrolo[2,1-*c*][1,4]benzodiazepines of formula IX as given above where n is 3 to 10 which comprises reacting (2*S*)-N-[4-hydroxy-5-methoxy-2-nitrobenzoyl]-4-fluoro-2-carboxaldehyde diethylthioacetal of formula VI with a dibromoalkane in an aprotic water miscible organic solvents. The solvent is preferably chosen from acetone, acetonitrile, and DMF. The reaction is also carried out in the presence of a mild inorganic bases such as K_2CO_3, $CsCO_3$ and $BaCO_3$ and up to refluxing temperature for a period of 48 hours. The 1,1′ -{[(alkane-1,N-diyl)dioxy}bis[(2-nitro-5-methoxy-1,4-phenylene) carbonyl] bis [4-fluoropyrrolidin-2-carboxaldehyde diethylthioacetal] of formula VII formed where n is 3-10 is then isolated by conventional methods and reduced with $SnCl_2 .2H_2O$ in presence of organic solvent up to a reflux temperature. The 1,1'-{[(alkane-1,N-diyl)dioxy}bis[(2-amino-5-methoxy-1,4-phenylene)carbonyl]]bis [4-fluoro-pyrrolidin-2-carboxaldehyde diethylthioacetal]] of formula VIII formed where n is 3-10 is then isolated by known methods. The compound of formula VIII is then reacted with a known deprotecting agent in a conventional manner to obtain the novel bis 2-fluoro pyrrolo[2,1-c][1,4]benzodiazepines of formula IX wherein n are as stated above.

In the alternate, the process comprises first reacting methyl (2*S*)-N-[4-benzyloxy-5-methoxy-2-nitrobenzoyl]-4-hydroxypyrrolidine-2-carboxylate dissolved in an organic solvent such as CH_2Cl_2 and cooling the solution to -78°C. To this cooled solution, a solution of diethylaminosulfurtrifluoride (DAST) in an organic solvent such as CH_2Cl_2 is added drop wise over a period of 40 min. After 15 hours methyl (2*S*)-N-

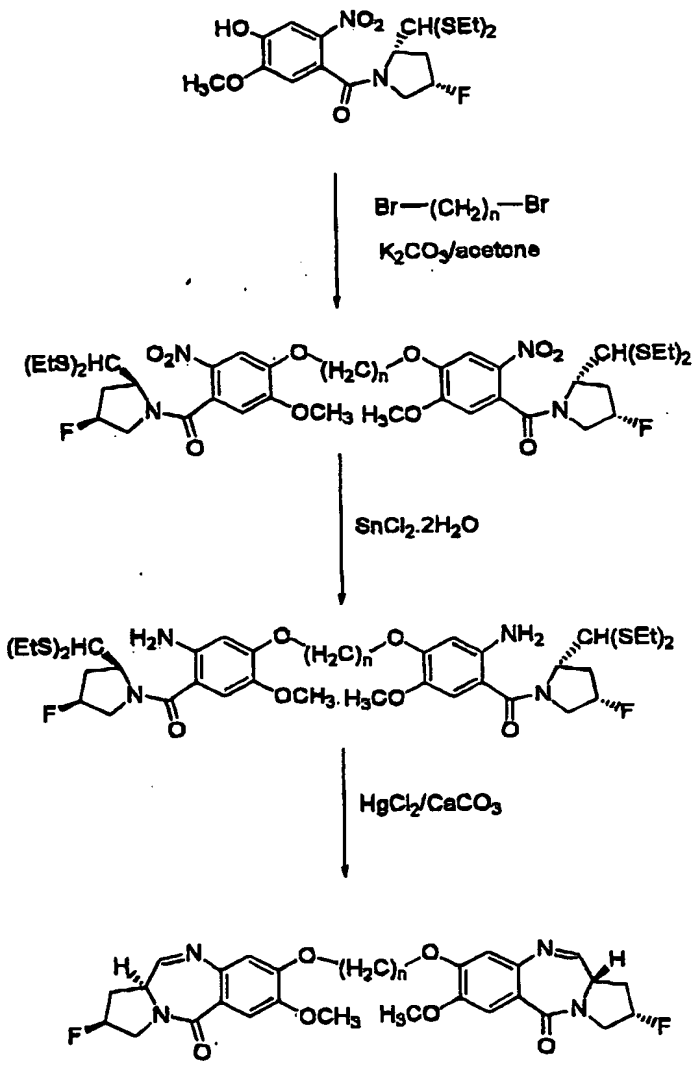
[4-benzyloxy-5-methoxy-2-nitrobenzoyl]-4-fluoropyrrolidine-2-carboxylate of formula II with DIBAL-H formula III is isolated and then cooled in the presence of organic solvent such as CH_2Cl_2 -78°C for a period of 45 min. The methyl (2*S*)-N-[4-benzyloxy-5-methoxy-2-nitrobenzoyl]-4-fluoropyrrolidine-2-carboxaldehyde formed is isolated by conventional methods and protected with EtSH in the presence of organic solvent at room temperature. The (2*S*)-N-[4-benzyloxy-5-methoxy-2-nitrobenzoyl]-4-fluoropyrrolidine-2-carboxaldehyde diethylthioacetal obtained is then isolated by known methods and reacted with any conventional debenzylating agent to give (2*S*)-N-[4-hydroxy-5-methoxy-2-nitrobenzoyl]-4-fluoropyrrolidine-2-carboxaldehyde-diethylthioacetal of formula VI. The compound of formula VI is then converted to the compound of formula IX in the manner indicated above.

The precursor, methyl (2*S*)-N-(4-benzyloxy-5-methoxy-2-nitrobenzoyl)-4-hydroxypyrrolidine-2-carboxylate (intermediates of DC-81) was prepared by literature methods (Thurston, D.E.; Murthy, V. S.; Langley, D. R.; Jones, G. B. *Synthesis*, 1990, 81.)

Some representative compounds of formula IX of present invention are given below:

1) 1,1'-{[(propane-1,3-diyl)dioxy]bis[(11a*S*)-2-fluoro-7-methoxy-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one]}
2) 1,1'-{[(butane-1,4-diyl)dioxy]bis[(11a*S*)-2-fluoro-7-methoxy-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one]}
3) 1,1'-{[(pentane-1,5-diyl)dioxy]bis[(11a*S*)-2-fluoro-7-methoxy-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one]}

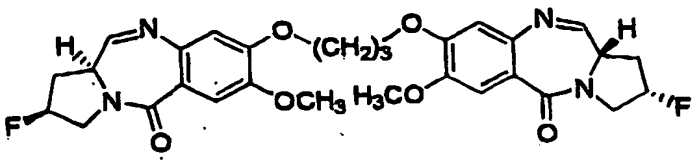
The reaction scheme is given below:



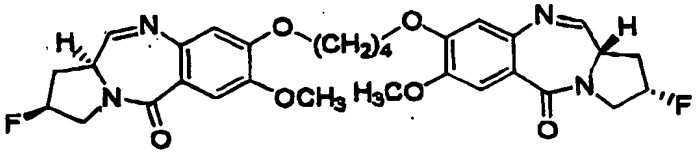
These new analogues of pyrrolo[2,1-c][1,4]benzodiazepine dimers substituted at C-2 position have shown promising anticancer activity in various cell lines. The molecules synthesized are of immense biological significance with potential sequence selective DNA-binding property. This resulted in design and synthesis of new congeners, which comprise:

1. The fluoro substitution at C-2 position of DC-81 intermediates.
2. The ether linkage between two fluoro DC-81 monomers at C-8 position.
3. Refluxing the reaction mixture for 24-48 h.
4. Synthesis of fluoro PBD antitumour antibiotic dimer imines.
5. Purification by column chromatography using different solvents like ethylacetate, hexane, dichloromethane and methanol.

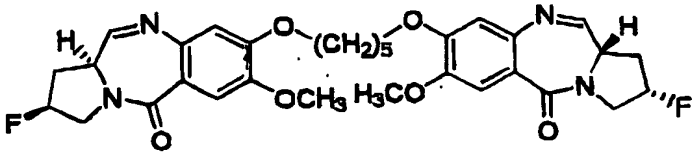
Representative compounds of Formula IX include



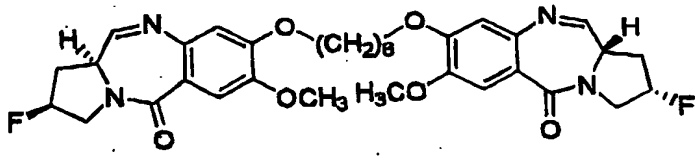
Formula IXa



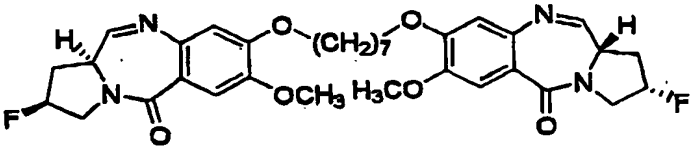
Formula IXb



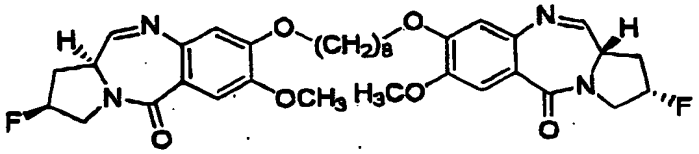
Formula IXc



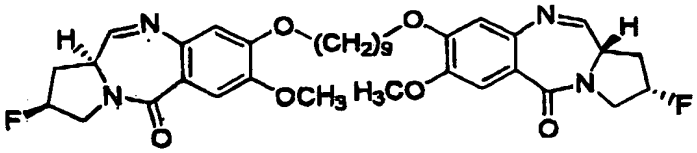
Formula IXd



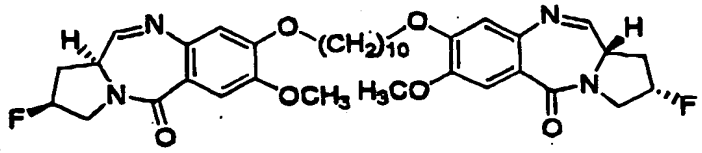
Formula IXe



Formula IXf.



Formula IXg



Formula IXh

The following examples are given by way of illustration and therefore should not be construed to the present limit of the scope of invention.

Example 1

A solution of (2S)-N-(4-hydroxy-5-methoxy-2-nitrobenzoyl)-4-fluoro pyrrolidine 2-carboxaldehyde diethylthioacetal VI (418 mg, 1 mmol), 1, 3-dibromopropane (101 mg, 0.5 mmol) and K_2CO_3 (414 mg, 3 mmol) in dry acetone (40 ml) was refluxed for 48h. After the completion of reaction as indicated by TLC, EtOAc-hexane (7:3), the reaction mixture was poured on to the water and then extracted with ethylacetate. Evaporation of the organic layer gave the crude product, which was further purified by column chromatography on silica gel eluting with EtOAc-hexane (1:1) to give the pure 1,1'-{[(Propane-1,3 diyl)dioxy]bis[2-nitro-5-methoxy-1,4-phenylene) carbonyl]}bis[4-fluoro pyrrolidine-2-carboxaldehyde diethylthioacetal] VII. H^1 NMR ($CDCl_3$, 200 MHz): δ 1.2-1.39 (m, 12H), 2.4-2.68 (m, 6H), 2.7-2.9 (m, 8H), 3.41-3.62 (m, 4H), 3.99 (s, 6H), 4.29-4.4 (m, 4 H), 4.52 (d, J = 3.9 Hz, 2H), 4.69-4.79 (m, 2H), 5.05 (t, 1H), 6.85 (s, 2H), 7.63 (s, 2H). FAB MASS: 877 (M+H)

1,1'-{[(Propane-1,3diyl)dioxy]bis[2-nitro-5-methoxy-1,4-phenylene) carbonyl]}bis[4-fluoro pyrrolidine-2-carboxaldehyde diethyl thioacetal] VII (876 mg, 1.0 mmol) was dissolved in methanol (10 mL) and to this was added $SnCl_2.2H_2O$ (1.124 g, 5.0 mmol) and was refluxed for 1.5 h. The reaction mixture was then carefully adjusted to pH 8 with saturated $NaHCO_3$ solution and then extracted with ethyl acetate (3x20 mL). The combined organic phase was dried over Na_2SO_4 and evaporated under vacuum to afford the crude 1,1'-{[(Propane-1,3 diyl)dioxy]bis[2-amino-5-methoxy-1,4-phenylene) carbonyl]}bis[4-fluoro pyrrolidine-2-carboxaldehyde diethyl thioacetal].

A solution of the 1,1'-{[(Propane-1,3 diyl)dioxy]bis[2-amino-5-methoxy-1,4-pheny-
lene) carbonyl]}bis[4-fluoro pyrrolidine-2-carboxaldehyde diethylthioacetal] VIII (846 mg, 1 mmol), $HgCl_2$ (794 mg, 2.93 mmol) and HgO (686 mg, 3.18 mmol) in CH_3CN/H_2O (3:1, 15 ml) was stirred at room temperature for 12h until TLC (EtOAc), indicated complete loss of starting material. Then organic layer is evaporated in vacuum and the residue is diluted with EtOAc. To this, saturated $NaHCO_3$ solution was

added slowly at room temperature and the mixture was filtered through celite and washed with ethylacetate. The filtrate was evaporated in vacuum to get crude 1,1'-{[(propane-1,3-diyl)]dioxy}bis[(11a*S*)-2-fluoro-7-methoxy-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4] benzodiazepin-5-one] of formula IXa, which was further purified by column chromatography on silica gel eluting first with ethyl acetate to remove traces of mercuric salts and further eluted with $CHCl_3$-methanol (8.5:1.5). H^1 NMR ($CDCl_3$, 200 MHz): δ 2.15-2.45 (m, 6H), 3.7-3.9 (m, 6H), 4.01(s, 6H), 4.22-4.3 (m, 4 H), 5.05 (t, 1H), 5.20 (t, 1H), 6.80 (s, 2H), 7.42 (s, 2H), 7.80 (d, 2H, J = 4.2 Hz). FAB MASS: 569 (M+H)

Example 2

A solution of (2S)-N-(4-hydroxy-5-methoxy-2-nitrobenzoyl)-4-fluoro pyrrolidine 2-carboxaldehyde diethylthioacetal VI (418 mg, 1 mmol), 1,4-dibromobutane (107 mg, 0.5 mmol) and K_2CO_3 (414 mg, 3 mmol) in dry acetone (20 ml) was refluxed for 48h. After the completion of reaction as indicated by TLC, EtOAc-hexane (7:3), the reaction mixture was poured on to the water and then extracted with ethyl acetate. Evaporation of the organic layer gave the crude product, which was further purified by column chromatography on silica gel eluting with EtOAc-hexane (1:1) to give the pure1,1'-{[Butane-1,4-diyl)dioxy]bis(2-nitro-5-methoxy-1,4-phenylene) carbonyl]}bis[4-fluoro pyrroilidine-2-carboxaldehyde diethylthioacetal] VII. H^1 NMR ($CDCl_3$, 200 MHz): δ 1.29-1.4 (m, 12H), 2.1-2.2 (m, 4H), 2.49-2.61 (m, 4H), 2.7-2.9 (m, 8H), 3.4 - 3.7 (m, 4H), 3.92 (s, 6H), 4.27 (t, 4 H), 4.58 (d, 2H), 4.70-4.85 (m, 2H), 5.08 (t, 1H), 5.29 (t, 1H), 6.82 (s, 2H), 7.65 (s, 2H). FAB MASS: 891 (M+H)

1,1'-{[Butane-1,4-diyl)dioxy]bis(2-nitro-5-methoxy-1,4-phenylene) carbonyl]} bis[4-fluoro pyrrolidine-2-carboxaldehyde diethylthioacetal] of formula VII (890 mg, 1.0 mmol) was dissolved in methanol (10 ml) and added $SnCl_2.2H_2O$ (1.124 g, 5.0 mmol) was refluxed for 1.5 h. The reaction mixture was then carefully adjusted to pH 8 with saturated $NaHCO_3$ solution and then extracted with ethyl acetate (3x20 ml). The combined organic phase was dried over Na_2SO_4 and evaporated under vacuum to afford the crude of pure1,1'-{[Butane-1,4-diyl)dioxy]bis(2-amino-5-methoxy-1,4-phenylene) carbonyl]}bis[4-fluoropyrrolidine-2-carboxaldehyde diethylthioacetal of formula VIII.

A solution of 1,1'-{[Butane-1,4-diyl)dioxy]bis(2-amino-5-methoxy-1,4-phenylene) carbonyl]}bis[4-fluoropyrrolidine-2-carboxaldehyde diethylthioacetal of formula VIII (861 mg, 1 mmol), $HgCl_2$ (794 mg, 2.93 mmol) and HgO (687 mg, 3.18 mmol) in CH_3CN/H_2O (3:1, 15 ml) was stirred at room temperature for 12h until TLC (EtOAc), indicated complete loss of starting material. Then organic layer was evaporated in vacuum and the residue is diluted with EtOAc. To this, saturated $NaHCO_3$ solution was added slowly at room temperature and the mixture is filtered through celite and washed with ethyl acetate. The filtrate was evaporated in vacuum to get crude 1,1'-{[(butane-1,4-diyl)]dioxy}bis[(11a*S*)-2-fluoro-7-methoxy-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4] benzodiazepin-5-one IXb, which was further purified by column chromatography on silica gel eluting first with ethylacetate to remove traces of mercuric salts and further eluted with $CHCl_3$-methanol (9:1). H^1 NMR ($CDCl_3$, 200 MHz): δ 1.94-2.09 (m, 4H), 2.1-2.5 (m, 4H), 3.5 - 3.82 (m, 6H), 3.98 (s, 6H), 4.1-4.37 (m, 4H), 5.29 (t, 1H), 5.5 (t, 1H), 6.80 (s, 2H), 7.45 (s, 2H), 7.80 (d, 2H, J = 4.3 Hz). FAB MASS: 583 (M+H)

Example 3

A solution of (2S)-N-(4-hydroxy-5-methoxy-2-nitrobenzoyl)-4-fluoro pyrrolidine 2-carboxaldehyde diethylthioacetal VI (418 mg, 1 mmol), 1,5-dibromopentane (114 mg, 0.5 mmol) and K_2CO_3 (414 mg, 3 mmol) in dry acetone (20 ml) was refluxed for 48h. After the completion of reaction as indicated by TLC, EtOAc-hexane (7:3), the reaction mixture was poured on to the water and then extracted with ethylacetate. Evaporation of the organic layer gave the crude product, which was further purified by column chromatography on silica gel eluting with EtOAc-hexane (1:1) to give the pure1,1'-{[Pentane-1,5-diyl)dioxy]bis(2-nitro-5-methoxy-1,4-phenylene) carbonyl]}bis[4-fluoro pyrroilidine-2-carboxaldehyde diethyl thioacetal VII. H^1 NMR ($CDCl_3$, 200 MHz): δ 1.2-1.42 (m, 12H), 1.65-2.1 (m, 6H), 2.4 - 2.61 (m, 4H), 2.7-2.91 (m, 8H), 3.29-3.67 (m, 4H), 3.99 (s, 6H), 4.09-4.25 (m, 4H), 4.52-4.68 (m, 2H), 4.82 (d, 2H), 5.10 (t, 1H), 5.32 (t, 1H), 6.89 (s, 2H), 7.69 (s, 2H). FAB MASS: 905 (M+H)

1,1'-{[Pentane-1,5-diyl)dioxy]bis(2-nitro-5-methoxy-1,4-phenylene) carbonyl]} bis[4-fluoro pyrroilidine-2-carboxaldehyde diethylthioacetal].of formula VII (905 mg, 1.0 mmol) was dissolved in methanol (10 ml) and to it was added $SnCl_2.2H_2O$ (1.124 g, 5.0 mmol) and was refluxed for 1.5 h. The reaction mixture was

then carefully adjusted to pH 8 with saturated $NaHCO_3$ solution and then extracted with ethyl acetate (3x20 ml). The combined organic phase was dried over Na_2SO_4 and evaporated under vacuum to afford the crude 1'-{[Pentane-1,5-diyl)dioxy]bis(2-amino-5-methoxy-1,4-phenylene)carbonyl]}bis[4-fluoropyrrolidine-2-carboxaldehyde diethyl thioacetal.of formula VIII.

A solution of 1,1'-{[Pentane-1,5-diyl)dioxy]bis(2-amino-5-methoxy-1,4-phenylene) carbonyl]}bis[4-fluoropyrrolidine-2-carboxaldehyde diethylthioacetal of formula VIII (875 mg, 1 mmol), $HgCl_2$ (794 mg, 2.93 mmol) and HgO (687 mg, 3.18 mmol) in CH_3CN/H_2O (3:1, 15 ml) was stirred at room temperature for 12h until TLC (EtOAc) indicated complete loss of starting material. Then organic layer was evaporated in vacuum and the residue was diluted with EtOAc. To this, saturated $NaHCO_3$ solution was added slowly at room temperature and the mixture was filtered through celite and washed with ethylacetate. The filtrate was evaporated in vacuum to get crude 1,1'-{[(pentane-1,5-diyl)]dioxy}bis[(11a*S*)-2-fluoro-7-methoxy-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4] benzodiazepin-5-one IXc, which was further purified by column chromatography on silica gel eluting first with ethylacetate to remove traces of mercuric salts and further eluted with $CHCl_3$-methanol (9:1). H^1 NMR ($CDCl_3$, 200 MHz): δ 1.58-1.81 (m, 4H), 1.90-2.01 (m, 2H), 2.38 - 2.50 (m, 4H), 3.08-3.24 (m, 4H), 4.01-4.20 (m, 4H), 4.92 (s, 6H), 5.21 (t, 1H), 5.5 (t, 1H), 6.81 (s, 2H), 7.49 (s, 2H), 7.83 (d, 2H, J = 4.4 Hz). FAB MASS: 597 (M+H)

Biological Activity: *In vitro* biological activity studies were carried out at National Cancer Institute (USA).

Cytotoxicity: Compounds IXa and IXc were evaluated for *in vitro* against sixty human tumour cells derived from nine cancer types (leukemia, non-small-cell lung, colon, CNS, melanoma, ovarian, prostate, and breast cancer). For each compound, dose response curves against each cell line were measured at a minimum of five concentrations at 10 fold dilutions. A protocol of 48 h continuous drug exposure was used and a sulforhodamine B (SRB) protein assay was used to estimate cell viability or growth. The concentration causing 50 % cell growth inhibition (GI50), total cell growth inhibition (TGI, 0% growth) and 50% cell death (LC50, -50% growth) compared with the control was calculated. The mean graph midpoint values of log_{10}TGI and log_{10}LC50 as well as log_{10} GI50 for IXa and IXc are listed in **Table 1**. As demonstrated by mean graph pattern, compound IXc exhibits an interesting profile of activity and

selectivity for various cell lines. The mean graph mid point of log_{10} TGI and log_{10} LC50 showed similar pattern to the log_{10} GI50 mean graph mid points.

Table 1. log_{10} GI50 log_{10} TGI and log_{10} LC50 mean graphs midpoints (MG_MID) of *in vitro* Cytotoxicity data for the compounds IXa and IXc against human tumour cell lines.

Compound	Log_{10} GI50	Log_{10} TGI	Log_{10} LC50
IXa	-5.21	-4.75	-4.31
IXc	-7.14	-6.27	-4.87

Table 2. *In vitro* one dose primary anticancer assay[a] bisfluorinated PBDs of formula IXa, and IXc

PBD	Growth percentages		
	(Lung) NCI-H460	(Breast) MCF7	(CNS) SF-268
IXa	0	0	0
IXc	0	0	0

[a]One dose of IXa and IXc at 10^{-4} molar concentration

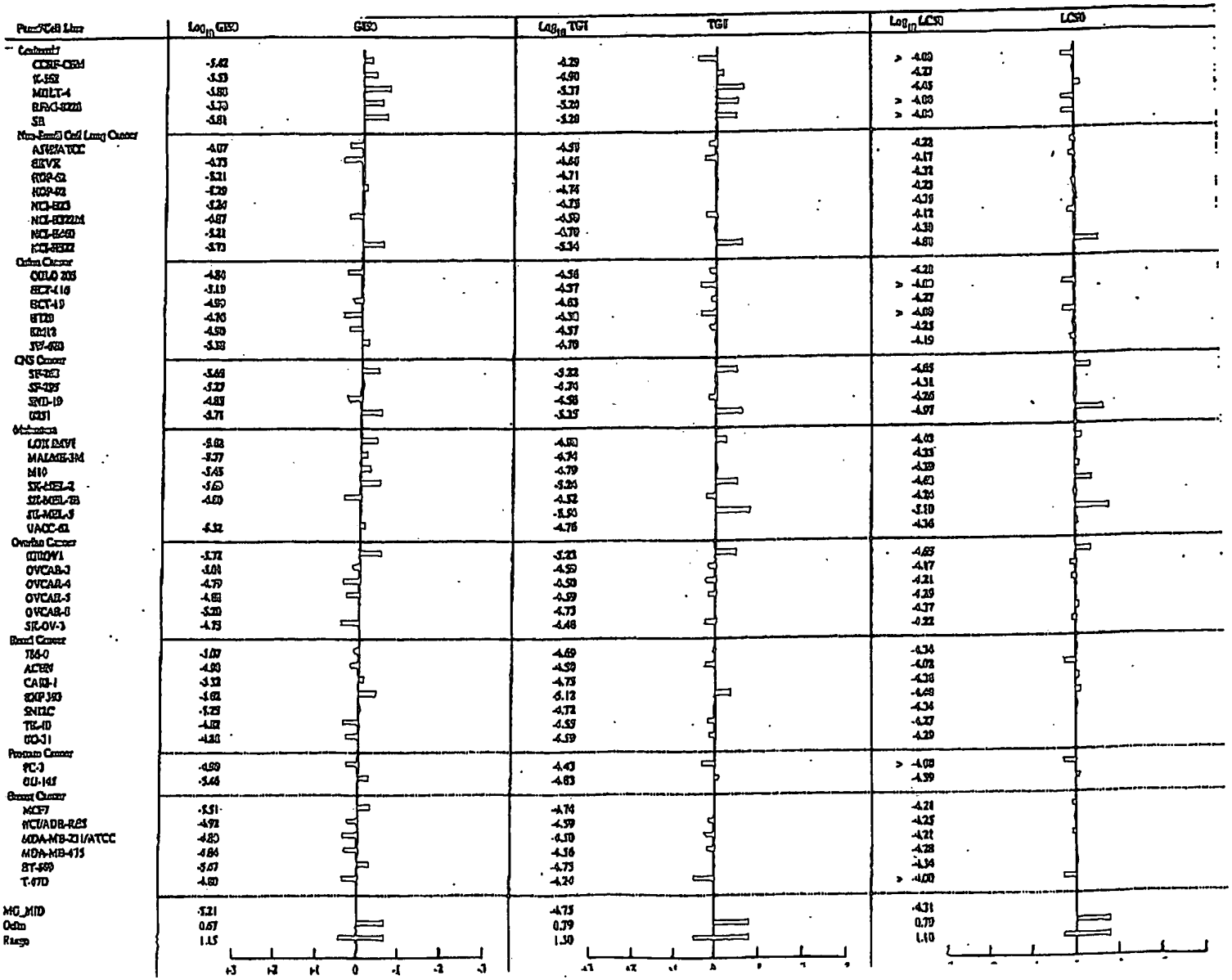
The anticancer activity for two representative compounds has been given in **Table 2**. The comparison of the data of **Table 3** reveals the importance of the alkane spacer. As the alkane spacer increased from 3-5 the cytotoxic activity has moderately enhanced. The 5 carbon spacer of compound IXc confers a suitable fit in the minor groove of double helix DNA and shows slightly higher activity in this series of compounds IXa and IXc.

Table 3. Log GI50 (inhibitory concentration) Values for Compounds IXa, and IXc

cancer	Compound IXa	Compound IXc
leukemia	5.668	7.794
non-small-cell lung	5.258	7.318
colon	5.285	7.064
CNS	5.543	7.625
melanoma	5.490	7.301
ovarian	5.310	6.620
renal	5.315	7.492
prostate	5.180	7.430
breast	5.490	7.234

Each cancer type represents the average of six to eight different cancer cell lines.

MEAN GRAPH TABLE 4



MEAN GRAPH TABLE 4